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Abstract

Background: Cheese manufacturing consists of a dehydration process by which most of the whey is drained off. Whey proteins (WP) represent a nutritionally valuable fraction largely lost in the whey stream. The increasing consumer demand for food with novel texture and functionality prompted the dairy industry to exploit the valorization of WP. Scope and approach: Cheese milk is considered a vehicle for the inclusion of WP. In this review, we discuss technological tools to fortify cheese with WP. We focus on both the pioneer approaches (the heat treatment of cheese milk, membrane separation technologies and the direct addition of milk- or whey-based derivatives) and on more recent techniques such as high hydrostatic pressure, ultra-high pressure homogenization, transglutaminase treatment of cheese milk and direct addition of buttermilk. Key findings and conclusions: Milk fortification with WP influences the yield and overall properties of resulting cheeses. Processing conditions need to be adapted to successfully manufacture WP-fortified conventional cheeses as well as novel varieties (such as low-fat cheeses). As yet, only some technological tools to include WP in cheese are exploited at industrial scale. The combination of different techniques is a strategy to overcome the impairment of cheese attributes.

Keywords	whey proteins; fortification; cheese; yield; whey-based derivatives.
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January 24th, 2017

Dear Editor,

On behalf of all Authors, I have a pleasure to submit the manuscript “Technological tools to include whey proteins in cheese: current status and perspectives” by Stefano Cattaneo, Milda Stuknytė, Ivano De Noni and myself to be considered for publication in *Trends in Food Science & Technology*. In this review, we discuss the progress in technological approaches useful to enhance the recovery of whey proteins in cheese. Focus is addressed both to recent developments in research and to strategies based on the combination of multi-step processes. In our opinion, this topic in dairy technology captures the interest of *i)* researchers for a better knowledge of the comparative quality of cheeses, *ii)* consumers for their expectations in foods with novel texture and functionality and *iii)* cheese makers for the subsequent increase in cheese yield.

We slightly exceeded the limit of 10,000 words due to the large amount of data collected in tables.

We hope that the manuscript could be considered for the publication in *Trends in Food Science & Technology*.

Sincerely,
Fabio Masotti

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TECHNOLOGICAL TOOLS TO INCLUDE WHEY PROTEINS IN CHEESE: CURRENT STATUS AND PERSPECTIVES

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ABSTRACT

Background: Cheese manufacturing consists of a dehydration process by which most of the whey is drained off. Whey proteins (WP) represent a nutritionally valuable fraction largely lost in the whey stream. The increasing consumer demand for food with novel texture and functionality prompted the dairy industry to exploit the valorization of WP.

Scope and approach: Cheese milk is considered a vehicle for the inclusion of WP. In this review, we discuss technological tools to fortify cheese with WP. We focus on both the pioneer approaches (the heat treatment of cheese milk, membrane separation technologies and the direct addition of milk- or whey-based derivatives) and on more recent techniques such as high hydrostatic pressure, ultra-high pressure homogenization, transglutaminase treatment of cheese milk and direct addition of buttermilk.

Key findings and conclusions: Milk fortification with WP influences the yield and overall properties of resulting cheeses. Processing conditions need to be adapted to successfully manufacture WP-fortified conventional cheeses as well as novel varieties (such as low-fat cheeses). As yet, only some technological tools to include WP in cheese are exploited at industrial scale. The combination of different techniques is a strategy to overcome the impairment of cheese attributes.

Keywords: Whey proteins; Fortification; Cheese; Yield; Whey-based derivatives.

Introduction

Cheesemaking is the process by which milk components present as colloidal dispersion (casein (CN) and calcium phosphate) and emulsion (fat) form a network, while the soluble constituents (whey proteins (WP), lactose and some minerals) are largely drained off in the whey. More than half of solids of the whole milk are contained in whey, making it stand out as a useful resource of nutrients. About 80 to 90% in volume of milk-in-vat is recovered as whey after cheesemaking. The management of this by-product has been a topic of interest for a long time, due to the increasing ongoing volumes worldwide, the high organic content and environmental- and health-related issues. Extensive investigations focused on the exploitation of technofunctional, biological and nutritional properties of the whey (Guyomarc'h et al., 2015; Yadav et al., 2015). By far, membrane technology enabled the breakthrough of whey processing into several derivatives favoring their incorporation as ingredients into different foods. WP are perceived as high-quality proteins, and for this reason, their application field covers different food and dairy industry sectors. Cheese represents an attractive delivery vehicle for protein-based ingredients due to the simultaneous increase of yield (Yadav et al., 2015) and the widespread consumption. The relevance of this topic, going far back in time, was demonstrated by the pioneering development of ultrafiltration (UF) techniques on behalf of dairy industry. The target to make cheeses with ultrafiltered milk consists in the retention or the reintegration of WP to increase the yield of the end product. Fortification of cheese with WP to boost its nutritional profile has been identified as an additional target with a steadily growing trend. Nutritional investigations on high-protein diet evidenced the considerable value of whey-derived protein sources because of their metabolic influence on weight management and body composition (Pichon et al., 2008). WP and CN differ markedly in terms of amino acid composition and behavior during digestion and absorption in the intestine. CN is relatively rich in tyrosine and phenylalanine, while WP in sulfur-containing amino acids, lysine, threonine and tryptophan (Pellegrino, Masotti, Cattaneo, Hogenboom, & De Noni, 2013). More recently, Singh, Øiseth, Lundin, and Day (2014) highlighted that processing conditions (shearing) applied to WP promote structural changes affecting their digestibility. Such knowledge could have an outcome in the release of biologically active peptides.

Different pre-treatments of cheese milk have been developed to incorporate native or denatured WP in the cheese matrix. These options opened new avenues for progress in cheesemaking. However, several issues related to the inclusion of WP in cheese and contributing to scale-up capability should be evaluated (Fig. 1). The inclusion of WP in conventional cheese is a theoretically viable strategy, because according to the definition adopted by the Codex Alimentarius Commission (Codex, 2011), cheese is defined as the product in which the whey protein/casein ratio does not exceed that of milk. However, the slow growth rate at industrial level of a technique for the enhancement of WP content in cheese is partly ascribable to the uncertainty of its use in compliance with regulations and the varying state laws.

This review focuses on novel insights and recent developments reported in literature on the processing techniques to increase the recovery of WP in cheese. We surveyed approaches to verify their feasibility alone or in combination, pointing out benefits and drawbacks in terms of overall quality attributes of the deriving cheeses.

Technologies to include whey proteins in cheese

Figure 2 illustrates cheese milk pre-treatments targeted toward the inclusion of WP in cheese and characterized by different viability at cheese factory and variable effectiveness in WP recovery. We reported the current literature on these technological tools, including the influence on the yield and the overall quality of resulting cheeses, in Tables 1 and 2. The strategy based on the combination of several cheese milk pre-treatments to recover WP in cheese could be successfully exploited to overcome cheese defects subsequent to the adoption of a single-step procedure (Table 3). Finally, we detailed a synoptic presentation of the technological tools herein reviewed with advantages and drawbacks in terms of cheese quality in Table 4.

Heat treatment of milk

The influence of heating on cheesemaking properties of milk has been an issue of large interest for research studies (Singh & Waungana, 2001). Differently from CN, which is a heat-stable protein, WP starting from about

60 °C denature in a temperature/time-dependent manner (Guyomarc'h, 2006). Conventional pasteurization (72 °C for 20 s) triggers only 2 to 5% denaturation of WP (Walstra, Wouters, & Geurts, 2006). In detail, WP unfold, denature and, eventually, aggregate via thiol-disulfide interchange reactions and hydrophobic or electrostatic interactions. Reactive thiol groups of ß-lactoglobulin (ß-Lg) can self-aggregate or interact with κ-CN forming both soluble and micelle-bound complexes (Donato & Guyomarc'h, 2009), similar in the appearance to filamentous appendages protruding from the surface of the micelles (Fox, Guinee, Cogan, & McSweeney, 2017). The *para*-κ-CN/ß-Lg network is characterized by reduced syneretic properties. Thus, in addition to the incorporation of denatured WP in the curd, also an increase in moisture contributes to the improvement of the cheese yield (Kelly, Huppertz, & Sheehan, 2008). Provided cheese quality is maintained, milk heating is considered an economically profitable way to increase the cheese yield (Fox et al., 2017). The use of pasteurized milk at 72 °C for 15 s promotes only a fractional increase in cheese yield. However, too severe WP denaturation of cheese milk excessively prolongs rennet coagulation time and, as a consequence, weaker and finer curds form, retaining more water than normal (Huppertz et al., 2005; Singh & Waungana, 2001). Cheeses show a crumbly and soft texture with poor meltability (Guyomarc'h, 2006). Generally, clotting and syneresis can be satisfactory, upon adjusted cheesemaking conditions, up to about 20% WP denaturation. Under these conditions, protein yield can be increased by about 4% (Walstra et al., 2006). Atasoy, Yetişmeyen, Türkoğlu, and Özer (2008) evaluated the influence of heat treatment of cheese milk on the properties of Urfa cheese. By heating milk at 65 °C for 20 min or 75 °C for 5 min the cheese yield increased 3.5 and 9.9%, respectively. However, the higher temperature impaired chemical and sensory properties of the cheese. Hougaard, Ardö, and Ipsen (2010), applying instant infusion to cheese milk at temperatures of 72, 100 and 120 °C (with holding time of 0.2 s), recorded prolonged rennet coagulation time, reduced curd firming rate and increased the yield reflecting the intensity of heat treatment. Obtained cheeses differed from those deriving from raw or high-temperature short-time pasteurized milk in terms of composition, texture and ripening. Thermal treatment of cheese milk was proposed as a solution to limit or prevent undesirable rheological and textural characteristics typical of reduced-fat cheeses (Table 1).

An application of milk heating to increase cheese yield, enabling WP recovery, would result more appropriate in lactic or fresh cheeses (Donato & Guyomarc'h, 2009). Hinrichs (2001) reported that the increasing intensity of milk heating in fresh acid-cheese technology translated in a progressive increase in the retention of WP. Obtained curds were smoother than those achieved with conventional pasteurization process, due to the increased water solvation of denatured WP.

Literature suggested various modifications to overcome deleterious heat-induced changes, favoring WP retention into cheeses, namely:

i) to increase soluble calcium levels of milk by addition of $CaCl_2$ prior to renneting. This option was adopted in Cottage cheese to restore the cheesemaking properties of heated milk (Makhal, Kanawjia, & Giri, 2015). Milk heating at 90 °C for 5 min (plus 0.02% $CaCl_2$) enhanced the yield by about 17%, keeping the cheese quality of control cheese;

ii) to combine heat treatment of milk and membrane separation technology. The increase in protein concentration by microfiltration counteracts the thermal impact (see the following paragraph "Membrane processes").

iii) to use a mixture of raw/pasteurized milk and a minor amount of high-heated milk. This strategy, successfully adopted in soft cheeses, allows only a slight recovery of denatured WP in the cheese (Heino, Rauva, & Outinen, 2010), resulting in a drastic economic improvement in a large-scale process.

iv) to fortify milk with heat-denatured WP. In particular, addition of heat-denatured WP recovered from severely heated whey is an effective solution. This approach could be realized by adding a whey cheese, such as Ricotta, to milk-in-vat. Thomet, Bachmann, and Schafroth (2005) verified that the large flocks of WP in Ricotta remained entrapped into the coagulum creating a soft texture with an increased capacity to absorb water into the cheese. Ismail, Ammar, and El-Metwally (2011) reported this practice to be particularly suited to low-fat semi-hard cheeses.

v) to heat milk at alkaline pH. The progressive increase of the pH of heat treatment is paralleled by both an increase in the dissociation rate of κ-CN and the formation of aggregates of denatured WP and κ-CN in the serum phase rather than on surface micelles (Guyomarc'h, 2006). This effect minimizes deleterious consequences on the rennet coagulation properties of the milk.

Summing up, the recovery of WP by milk or whey heating is economically appealing, because it does not require additional equipment other than heat exchangers. Fresh acid and semi-soft cheeses do not require extensive drainage and the high-heat treatment of milk is a realistic and feasible approach for the enhancement of cheese yield by transfer of WP to the cheese matrix (Donato & Guyomarc'h, 2009), thus offering the advantage of a greater nutritional value. Differently, heated milks show reduced rennet coagulation properties. Corresponding curds appear soggy and ragged with poor stretching and melting properties (Singh & Waungana, 2001). In semi-hard and hard cheeses the consequence of the excessive inclusion of denatured WP would outcome in too high moisture of the cheese with the development of texture and flavor defects (Huppertz et al., 2005).

Membrane processes

Membrane processes consist of a pressure-driven separation and concentration of a feed through a membrane (Mistry & Maubois, 2004). The mechanism of separation is based on size and shape of molecules as well as on charge and affinity for the membrane. Milk and dairy industry have greatly benefited of membrane filtration technology for its effective and economic implementation. Among the classes of membrane techniques, differing for their molecular weight cut-off, UF was the first to be exploited for WP enrichment of cheese (Fox et al., 2017; Kelly et al., 2008). Basically, WP could be incorporated in cheese both in their native form (by their retention during processing) or in a denatured state (either after interaction with CN or as inert aggregates). In the first case, the cheese milk is directly submitted to UF, whereas the inclusion of denatured WP implies also the combined use of a heat treatment. The most commonly used UF retentates of cheese milk are as follows (Mistry & Maubois, 2004):

- *Low-concentration UF retentate* – Cheese milk is concentrated up to a maximum of 2-fold (generally, at a molecular cut-off $<2 \times 10^4$ g mol^{-1}), allowing to improve cheese yield by conventional cheese manufacturing techniques. Whey proteins, remaining entrapped in the curd matrix, contribute to the enhanced yield of cheese. Rennet-curd cheeses, such as Mozzarella, Cottage and Cheddar, can be manufactured by this technique (Guinee, O'Kennedy, & Kelly, 2006; Lipnizki, 2010). Agrawal and Hassan (2007) adopted this practice to make reduced-fat Cheddar cheese with the addition of strains of lactic acid bacteria producing exopolysaccharides to retain moisture, minimizing in this way some typical defects of low-fat cheeses (Table 1). Under the described conditions, the improved texture, melting and viscoelastic properties of the end product matched with the slightly higher WP content, which positively affected water-binding capacity;

- *Medium-concentration UF retentate* − Cheese milk concentration ranges from 2- to about 5-fold. WP preserved in the retentate are responsible for the increase in cheese yield. Lipnizki (2010) described the application of partially concentrated milk to make Queso Fresco, structure Feta, Camembert and Brie cheeses. Kelly et al. (2008) refer that the adoption of medium-concentration UF retentate is currently of little commercial interest;
- *High-concentration UF retentate or liquid pre-cheese* − Level of milk concentration is 6- to 7-fold, generally reaching the total solid content of the final cheese with the exception of harder varieties as their protocol requires a subsequent drainage (Fox et al., 2017). Following the adoption of this retentate in cheesemaking, a complete entrapment of WP into the cheese matrix is possible when further syneresis is not expexted. The use of liquid pre-cheese was assessed in different cheeses (soft or semi-hard rennet varieties) including Camembert, Blue cheese, Havarti and Mozzarella (Banks, 2007). An acceptable structure of the cheese was observed only when WP in the milk retentate were largely denatured by heat treatment. The so-called Cast Feta is made from liquid pre-cheese, acidified with starter culture and set with rennet. Both, cutting and drainage are not necessary, and the final cheese is characterized by the absence of conventional openings of Feta, with the advantage of greatly increased yield due to the retention of WP (Kethireddipalli & Hill, 2015). Lower UF retentates combined with heat treatment are preferred to obtain a more typical open-structured Feta. Six-fold concentrated UF milk allowed to increase the yield of industrial low-fat Camembert-like cheese (Hannon, Lortal, Tissier, & Famelart, 2009). However, resulting cheese showed several surface defects which were ascribed to a mineral layer acting as a barrier of lactate. The subsequent modification of the metabolism and the growth of surface moulds contributed to the reduced rate of ripening. More generally, to counteract this last drawback in UF-cheeses (promoted by extra WP as well as by the increase of buffering capacity), preserving their sensory quality,

milk acidification prior to UF can be adopted. Hannon, Lopez, Madec, and Lortal (2006) observed that pH reduction (from 6.6 to 5.2) of renneting in UF-cheese induced an earlier onset of the starter bacteria autolysis. Upon this process, intracellular enzymes were released into the cheese matrix and, as a consequence, the rate of cheese proteolysis increased.

Hinrichs (2001) reported different possibilities to extend the inclusion of WP in fresh acid cheese. The author suggested the addition to the curd of the UF retentate from the acid whey after heating. Such procedure allowed a large integration of WP (from 50 to 100%), as a function of the amount of added retentate. Meanwhile, excessive supplementation developed taste defects during cheese ripening. Another process to extend WP yield in fresh cheese consisted in the concentration of the heated cheese milk prior to fermentation. This option, although effective in terms of yield, resulted in the development of bitter taste as a consequence of the proteolytic activity of starter culture. This defect was overcome when UF process was applied to fermented curd. The prior acidification of the curd resulted in a fresh cheese with smooth taste.

Generally, a substantial benefit in cheese yield is gained by UF technology, in particular when applied to the heated cheese milk. Successful developments were recorded in soft varieties either fresh acid or ripened (Kethireddipalli & Hill, 2015). The ongoing investigations on this technology aim to enlarge the field of applications by proper adjustments and optimization of processing conditions. As a rule of thumb, a profitable application of UF technology to all cheeses requires a careful evaluation of the physicochemical properties of the retentate as they contribute to the UF-cheese quality and consequently to the consumer acceptance.

To a lesser extent than UF, microfiltration (molecular weight cut-off $>2 \times 10^5$) may be used to retain denatured WP into cheese as well. Thomet, Bachmann, and Schafroth (2004) coupled heat treatment and microfiltration of milk to recover heat denatured WP in Raclette-type cheese (Table 3). The authors observed a yield increase. However, suitable processing conditions were crucial to avoid cheese defects and to obtain a product with good melting properties. This strategy was applied in Gouda-type cheesemaking, too (Chromik, Partschefeld, Jaros, Henle, & Rohm, 2010). A significant amount of WP was retained by cheesemakng milk after microfiltration and subsequent heating (up to 85 °C for 60 s). Selected processing conditions allowed to reduce by 15 to 30% the loss of protein in the permeate.

High hydrostatic pressure treatment

High hydrostatic pressure (HHP) treatment of milk developed in recent years in comparison to heating. In depth studies on this non-thermal technology applied to milk and dairy products, evolved starting from the 1990s as an alternative tool to guarantee high safety, shelf life and reduced changes in quality (Chawla, Patil, & Singh, 2011). Several reports focused on the effects over yield and cheese properties (Table 1). High hydrostatic pressure treatment of milk triggers change in WP structure in a manner similar to thermal treatment. Huppertz, Fox, and Kelly (2004) reported that 90% of ß-Lg is denatured after treatment at 400 MPa for 30 min. Casein fraction is affected too, and extensive disruption of micelles occurs when the HHP exceeds 300 MPa, due to higher solubility of calcium phosphate. The authors observed that the denaturation of ß-Lg followed by the interaction with CN micelles is the major contributor to the enhanced cheese yield of HHP-treated milk.

Voigt, Donaghy, Patterson, Stephan, and Kelly (2010) reported that HHP-treated milks at 400 and 600 MPa (for 10 min at 20 °C) resulted in an increase in Cheddar yield of 1.2 and 7.8% in comparison to untreated cheese, respectively. Meanwhile, only a slight enhancement of moisture level (0.6 and 2.1%, respectively) was recorded. Differently, San Martín-González et al. (2007), carrying out pilot trials on Cheddar cheese from HHP-treated milk at 483 MPa, obtained a higher cheese yield (+ 11%) in comparison to control cheese from pasteurized milk and a similar increase in moisture (Table 1). The feasibility of HHP treatment (400 MPa for 20 min at 20 °C) of cheese milk was investigated also in Queso Fresco, a fresh white cheese (Sandra, Stanford, & Goddik, 2004). In this case, the entrapment of denatured WP raised the cheese yield as a consequence of their water-binding affinity. The cheese was less firm, less crumbly and stickier in comparison to the control from raw milk. The authors concluded that this milk pre-treatment represented an alternative to obtain Queso Fresco with lower microbial load, acceptable sensory attributes and similar composition to the conventional cheese from raw milk. Camembert cheese obtained from HHP-treated milk (500 MPa for 10 min at 20 °C) was characterized by high yield, extended denatured WP content and an accelerated hydrolysis of α_{s1}-CN in relation to the control from raw milk (Voigt, Patterson, Linton, & Kelly, 2011). The cheese was deemed similar by sensory evaluation to cheese from raw milk. However, further investigations were considered necessary to evaluate the effect on flavor pathways.

Overall, HHP pre-treatment of milk allows the increase of cheese yield with no detrimental consequences on coagulation properties and slight modifications of sensory attributes. However, numerous research studies on the effects of this technology in cheesemaking do not reflect the up-scale at industrial level in large dairies. The major drawback of the process is that it is discontinuous and time-consuming allowing the treatment of only limited milk quantities (Vannini et al., 2008). The size of the available processing units represents the industrial challenge. Nowadays, it is possible to treat up to 600 L in a semi-continuous process. To date, market applications have been launched only for treatment of some functional and fermented dairy products (Kelly et al., 2008). The high cost of processing is the other limit in comparison to the thermal treatment of cheese milk.

Ultra-high pressure homogenization

Ultra-high pressure homogenization (UHPH), also called dynamic high-pressure homogenization, is a technique of large interest in dairy sector (Dumay et al., 2013). This physical treatment differs from HHP as it is a continuous process, which combines homogenization and pressure effects. Its action mechanism is similar to that of conventional valve homogenizers except for the higher levels of pressure, reaching levels up to 400 MPa. Milk, submitted to UHPH treatment, passes throughout the homogenization valve and is exposed to high turbulence associated with some physical phenomena such as shear stress, hydrostatic pressure and cavitation (Escobar et al., 2011), which may be partly transformed into thermal energy. This phenomena reduce microbial load in milk, inactivate enzymes and modify functionality of milk proteins and fat. In addition, denaturation/aggregation of WP (as a function of the intensity of mechanical forces and/or temperature applied) is observed. UHPH enhances rennet coagulation properties of milk, by decreasing clotting time and increasing curd firming rate and curd firmness (Lodaite, Chevalier, Armaforte, & Kelly, 2009). The hyperbaric treatment of milk causes the retention of denatured WP in the curd as a function of the adopted homogenization conditions. The resulting cheese is characterized by increased yield and moisture retention. We listed recent applications of UHPH-treated milk as a means to increase WP retention and yield in cheese in Tables 1 and 3. Zamora, Ferragut, Juan, Guamis, and Trujillo (2011) and Zamora, Juan, and Trujillo (2015) observed that UHPH treatment of milk (300 MPa at 30 °C) triggered higher water retention and textural changes (i.e., higher firmness, lower deformability) of a starter-free cheese in comparison to the conventional variety from pasteurized milk (80 °C for 15 s). The poor cohesion of curd grains caused crumbling and improper curd matting during cutting as a consequence of the incorporation of CN and WP at the milk fat globule membrane following to the reduction of fat globule size.

A benefit of UHPH treatment of milk consists in the reduction of cheese ripening time, as proteolysis and lipolysis of milk are enhanced by naturally occurring or microbial enzymes. Milk pressurization at 100 MPa induced in Crescenza (an Italian soft rindless cheese) higher yield (1%) together with earlier and significant lipolysis, whereas proteolysis was very limited as in the conventional product from pasteurized milk (Lanciotti et al., 2004). Research studies on the effects of UHPH were carried out also with semi-hard varieties, such as Caciotta cheese (Lanciotti et al., 2006). An increase in yield (+ 23 and + 32% in comparison to cheeses obtained from pasteurized and raw milk, respectively) by retention of WP was observed, along with no detrimental effects on technological and sensorial attributes.

Heating and UHPH treatment of milk in the manufacture of Queso fresco resulted in higher yield and moisture content in comparison to the cheese from pasteurized milk alone (Escobar et al., 2011). Combined milk treatments promoted both thermal denaturation of WP and homogenization-induced dissociation of CN micelles. In this way, Queso fresco contained a thin CN-WP matrix capable of retaining more whey, so that avoiding the quality decay ascribable to excessive syneresis during ripening. UHPH technique (200 MPa at 30 °C) was also verified in goat milk to make semi-hard cheeses. Yield resulted + 3 and + 14% than that of cheeses obtained from homogenized-pasteurized milk (15 + 2 MPa and 72 °C for 15 s) and pasteurized milk (72 °C for 15 s), respectively (Juan, Zamora, Quevedo, & Trujillo, 2016). In ewe milk, the potential of UHPH treatment (100 MPa) was assessed in semi-hard Pecorino-like cheese (Vannini et al., 2008). The high retention of WP in the coagulum induced a sensitive increase of the yield (23 and 38% in comparison to the control cheese from thermized and raw milk, respectively). The accelerated ripening time with different proteolytic and lipolytic patterns characterized the cheese in comparison to the control. The authors concluded that UHPH treatment of milk contributed to the production of a new type of Pecorino-like variety with specific sensory characteristics.

The use of continuous or semi-continuous high-pressure homogenizers in cheesemaking is an encouraging and effective approach for yield increase, descending from the higher retention of WP in the curd and the improved water-binding capacity of proteins. Milk UHPH-treatment for the improvement of cheese quality or the development of novel dairy product varieties has not been yet implemented at industrial level. However, upscaling of the technology from laboratory-scale equipment to pilot-plant prototypes has been carried out.

Addition of dairy protein derivatives

Dairy protein derivatives are increasingly employed as milk extensions in cheese manufacture (Sharma, Jana, & Chavan, 2012). Main protein-based ingredients included for cheesemaking consist of skim milk powder (SMP), milk protein concentrate (MPC), WP concentrate (WPC) and microparticulated WP (MWP) (Fig. 2). Skim milk powder is a suitable ingredient for cheesemaking, being perhaps the most popular derivative used to improve the cheese yield (Lucey, 2015). However, the use of SMP can increase cheese browning and decrease pH due to the presence of residual sugars. Generally, MPC are preferred to enhance the cheese yield (Ur-Rehman, Farkye, Considine, Schaffner, & Drake, 2003). This dairy ingredient is identified by a protein content varying from 35 to 85% (Mistry & Maubois, 2004) and a CN to WP ratio equal to that of milk. Generally, the adoption of this fortification technique requires that specific attention is paid both to the proper amount of added MPC and to selected adaptations of cheesemaking process to avoid impairment of cheese properties. Several research studies assessed the influence of milk protein standardization with MPC to increase cheese yield and WP recovery (Table 2). In a previous work, by supplementing milk with variable amounts of WP sources (MPC or MWP), we observed an increase in the moisture of the deriving rennet-curds (Masotti, Cattaneo, Stuknytė, & De Noni, 2016). At the same time, we revealed a linear relationship between the amount of denatured WP added to cheese milk and that recovered in the curds.

Whey-based derivatives, are protein-enriched fractions exploitable for standardization of cheese milk and contributing to cheese yield. Research reports on this practice agree on: i) the preferred use of denatured WP to assure their entrapment in the cheese network, ii) the improved serum-holding capacity of the cheese, iii) the effect of WP aggregate dimensions on the attributes of the resulting fortified-cheese and iv) the flavor modifications in comparison to the derivative-free cheese. Jooyandeh (2009) investigated the combined effect of pre-acidification, starter culture concentration and WP incorporation on the texture of Iranian white cheese. The author tested the addition of fermented WPC to pre-acidify cheese milk prior to enzymatic coagulation. In this way, by removing some of the colloidal calcium, the cross-linking of CN was reduced leading to a cheese with higher moisture, softer texture and higher yield. However, excessive incorporation of fermented WPC (>15%) caused too soft structure of the cheese. Henriques, Gomes, Pereira, and Gil (2013), by adding different amounts of liquid denatured WPC (25 or 50 g per 100 g of milk proteins) to milk, obtained a fresh cheese with less pronounced reduction in yield during storage, in comparison to a control cheese supplemented with a CN derivative. This evidence was attributed to the improved water retention promoted by the entrapped WP aggregates. Sensorial parameters were modified and higher levels of added WPC reduced cheese acceptability.

Microparticulated WP have emerged over the last years as sources of WP with various functionalities. Currently, cheese makers can successfully exploit such ingredient. This derivative, in form of colloidal particles, consists of a mixture of native and aggregated denatured WP (Renard, Lavenant, Sanchez, Hemar, & Horne, 2002). Generally, to obtain MWP a WPC solution is firstly heated to denature WP and then physically sheared forming small protein aggregates. Microparticulated WP can differ for degree of denaturation, the shear applied during processing and the composition of the raw whey material (Skeie, Alseth, Abrahamsen, Jihansen, & Øyaas, 2013). Size and hydration of WP aggregates are crucial parameters controllable by physical and chemical means (Guyomarc'h, 2006). Particles with diameters ranging between 5 and 10 μm showed good results as additives in cheese enhancing the fatty-like mouthfeel. Above these values, the formation of cheese matrix is disturbed, resulting in reduced firmness and poor retention of WP (Suárez, Fernández, Balbarie, Iglesias, & Riera, 2016). These fillers act inertly, weakening the protein network and in this way lubricating the cheese matrix. However, the behavior of MWP is somewhat different from the simple non-interacting filling theory, Some components of MWP could interact with CN micelles, limiting gel formation and contraction (Perreault, Morin, Pouliot, & Britten, 2017).

As a general remark, addition of denatured MWP promotes an increase in the cheese yield by improved water-binding capacity and obstruction effect on whey drain-off. Hinrichs (2001) measured a positive relationship between the extent of WP denaturation of added MWP and the retention of WP in cheese. The reason of this phenomenon was ascribed to the fact that denatured and highly hydrated WP hindered syneresis. The recoverable amount of WP in MWP-fortified cheese is variable. Due to the higher moisture of the deriving cheese, this practice is advantageous in soft and semi-hard varieties. One challenge in the use of MWP is the optimization of their water-binding capacity. Actually, a limitation in the cheese milk supplementation with MWP is imposed by the consequent excessive cheese moisture. Saffon, Britten, and Pouliot (2011) proposed to circumvent this defect by including protein aggregates deriving from co-denaturation (pH 4.6, 90 °C) and homogenization (65.5 MPa) of cheese whey and buttermilk concentrates (Table 3). The relative increase of buttermilk amount up to a WP:buttermilk ratio of 25:75 reduced water holding capacity in the resulting cheese. In addition, the combination of ultrasound treatment (20 kHz) and heating of the mixture both increased the yield and decreased the water-holding capacity of aggregates. A further study explained the multi-step mechanism of the modified heat-induced denaturation of WP in presence of buttermilk (Saffon, Jiménez-Flores, Britten, & Pouliot, 2014). The rate of MWP addition to cheese milk represents a critical point. Indeed, excessive fortification adversely affects coagulation properties and quality of the cheese. Sturaro, Penasa, Cassandro, Varotto, and De Marchi (2014) revealed a significant increase in coagulation time when MWP addition exceeded 3.0%. A similar effect was observed for curd firming time, whereas curd firmness remained unaffected. The authors concluded with the necessity to adjust cheese processing (longer time for rennet activity) when MWP fortification was adopted.

Microparticulated WP, when added to milk as fat replacers in low-fat cheeses, not only enrich the cheese nutritionally due to inclusion of WP, but also improve its texture. Small aggregates of WP, remaining entrapped in the pores of the CN network, make the cheese softer. Including into milk increasing amounts (0.5, 1.0 or 2.0%) of commercial concentrated MPW, an increase in reduced-fat Gouda-type cheese yield was obtained, and its textural attributes were improved (Schenkel, Samudrala, & Hinrichs, 2011). In any case, the higher the concentration of MWP was, the lower resulted the firming rate. Such a drawback was overcome by adjusting processing technology. Low-fat Norvegia cheese (Gouda-type) with improved texture and flavor was obtained on pilot-plant scale, when buttermilk and different levels of liquid MWP (WP denaturation >85%) were included and selected adjunct cultures were implemented (Skeie et al., 2013). The cheese obtained by the recipe including buttermilk and 3% MWP in milk gave the highest liking scores. Differently, excessive addition of MWP (6%) was not beneficial for texture. Schenkel, Samudrala, and Hinrichs (2013) reported that MWP particles act as inert fillers within the *para*-κ-CN matrix in fat-reduced Gouda-type cheese. Addition of 1% denatured MWP (Simplesse®) to cheese milk improved thermo-rheological and texture attributes of the fortified cheese in comparison to the fat-reduced counterpart. Other studies evidenced similar results by exploring the effects of commercial MWP as fat replacers on the overall properties of different low-fat cheeses (soft and semi-hard) (Table 2).

The inclusion of denatured and aggregated WP to cheese milk represents a practical solution to satisfy consumer expectations in terms of texture and sensory attributes of fresh cheeses. A challenge to pursue is the optimization of the size and the water-binding capacity of these derivatives. Interaction mechanisms of added products with milk components upon processing, as well as the effect on overall cheese properties need further elucidation. This approach to include WP is preferable to heat treatment of milk due to the lower drawbacks in terms of curd-forming properties and cheese texture.

Addition of buttermilk and derivatives

Buttermilk is the aqueous phase released during destabilization of milk fat globules (churning) of cream in buttermaking. It contains components derived both from fragments of milk fat globule membrane (MFGM), mainly consisting of proteins and neutral as well as polar lipids, and all water-soluble components of cream (Vanderghem et al., 2010). However, buttermilk composition varies as a function of the source of origin and processing conditions adopted during buttermaking. In addition to sweet buttermilk (the main commercial type), also cultured buttermilk (in case of European-style buttermaking) and whey buttermilk (obtained from whey cream) are available (Sodini, Morin, Olabi, & Jiménez-Flores, 2006). This heterogeneity should be taken into account when buttermilk is used in cheesemaking. Main goal of milk fortification with buttermilk consists in the increase in yield provided cheese quality is unchanged. This result is reached through enhanced solvation, mainly ascribable to inclusion of phospholipids and denatured WP (Vanderghem et al., 2010). Industrially, buttermilk is separated from cream previously submitted to high pasteurization. Therefore, denatured WP interact with MFGM (Morin, Jiménez-Flores, & Pouliot, 2007). Furthermore, also the pasteurization of buttermilk, before drying, promotes a substantial denaturation of the membrane proteins and the consequent further association with residual WP (ß-Lg). Denatured WP attached to MFGM and retained in buttermilk are characterized by high water-binding capacity. Sweet buttermilk, condensed by heat and vacuum, supplemented at levels of 4 or 6% in regard to cheese milk improved the yield of pizza cheese with the contribution of denatured WP (Govindasamy-Lucey, Lin, Jaeggi, Johnson, & Lucey, 2006) (Table 2). Fortified pizza cheese melt and stretched less than control. In any case, only a slight effect on sensory attributes was observed by altering processing conditions. In addition, standardization of cheese milk with sweet buttermilk, concentrated by UF, increased moisture with no impairment of functional properties of pizza cheese (Govindasamy-Lucey et al., 2007). Morin, Pouliot, and Britten (2008) observed that cream heating prior to churning did not cause coagulation problems, when subsequently minute amounts of buttermilk were added to cheese milk. Differently, large substitution (50% of protein) of cheese milk adversely affected rennet gel properties through long cutting time, reduced firmness of the rennet gel and low contraction capacity. In this case, increasing percentages (up to 20%) of buttermilk led to softer and moister curd and acceptable sensory properties. Kumari, Kumar, Gupta, and Kumar (2012) verified the effect of buttermilk as ingredient in buffalo milk Chhana, an Indian-style soft cottage cheese analogue. By using excessive amounts of sweet buttermilk, the authors obtained an end product with undesired texture and sensory attributes. Changes in cheesemaking protocol were required to counteract the excessive increase of moisture responsible for texture defects. Substitution of milk with variable proportions of sweet buttermilk (from 0 to 50%) was technologically tested in cream cheese (Bahrami, Ahmadi, Beigmohammadi, & Hosseini, 2015). The authors revealed that the progressive increase of buttermilk percentage was followed by increase in moisture and yield. However, levels over 25% resulted in impaired sensory properties.

The valorization of buttermilk as cheese ingredient to improve quality attributes of reduced-fat cheeses has been investigated. Reduced-fat Paneer, an Asian soft cheese obtained by acid and heat coagulation of milk, made with different levels of sweet buttermilk (up to 40%) showed higher yields due to the moisture retention and binding of WP (Suneeta, Bhatt, & Prajapati, 2014). Addition of buttermilk in the range 20 to 30% improved sensory scores in comparison to the control and overcame the defects related to fat reduction.

In summary, buttermilk proves to be a valuable additional source of denatured WP in cheesemaking. However, a careful evaluation of the whole manufacturing procedure should be carried out not to adversely affect properties of the fortified cheese.

Transglutaminase treatment

The enzyme transglutaminase (EC 2.3.2.13) (TGase) has been widely investigated for its applications in dairy sector (Jaros, Partschefeld, Henle, & Rohm, 2006). The mode of action of TGase in milk consists in an acyl transfer reaction involving protein bound glutamyl and lysyl side chains and the subsequent formation of isopeptide bonds. In milk, CN are the preferred substrate of TGase, whereas native WP show a lower susceptibility due to their compact globular structure. Structural modifications, such as denaturation, make WP susceptible to the action of TGase (Jaros et al., 2006). WP denaturation by milk preheating before TGase incubation facilitates cross-link formation between CN and denatured WP. Transglutaminase reaction, negatively affecting rennetability of milk by extension of clotting time and reduction of gel firmness (Bönisch, Heidebach, & Kulozik, 2008), is less commonly experimented in semi-hard and hard cheesemaking (Aaltonen, Huumonen, & Myllärinen, 2014). To overcome the impairment of coagulation, the TGase treatment was proposed to be performed simultaneously or following the rennet action, resulting in yield increase due to covalently incorporated native WP in the cheese (Cozzolino et al., 2003; Di Pierro et al., 2010; Domagala et al., 2016). The co-addition of glutathione (a food grade reducing agent) and TGase to cheese milk simultaneously to rennet is the other alternative. Under these conditions, the TGase reaction promotes an enhanced water-holding capacity following the WP recovery in the coagulum. Bönisch et al., (2008) reported that the cheese yield increased from 11% for TGase-free curd to 20% for the curd obtained from milk renneted with a TGase (plus glutathione) at a concentration of 3.0 U per g protein. These authors supported a novel functionality of the gel network allowing manifold applications in the manufacture of soft and semi-hard cheeses. Özer, Hayaloglu, Yaman, Gürsoy, and Şener (2013), adopting the above mentioned reaction steps of cheese milk, proposed an effective tool for the manufacture of white brined cheese with increased moisture-adjusted yield (+ 9%) accompanied by modified texture (increased hardness). Generally, to pursue both yield increase and overall acceptability in semi-hard cheeses, TGase treatment of milk should be fully controlled by enzyme inactivation before cheesemaking. To this purpose, milk is heated up to 80 °C for 2 min. However, in this way WP denaturation impairs coagulation properties. A putative alternative procedure to the heat treatment is the addition of TGase inhibitor. In this way, the following rennet action is not affected. In this regard, Aaltonen et al. (2014) exploited TGase treatment for Edam cheesemaking in a controlled way. Ultrafiltered milk retentate (a TGase inhibitor-free substrate due to its low molecular weight) was submitted to TGase treatment. Subsequently, the enzyme was inactivated by diluting the retentate with raw milk (containing TGase inhibitor) in the standardization step. Following the cheesemaking, Edam yield increased (+ 4%) due to the moisture retention. Although the authors stated that TGase did not cross-link WP significantly, a slight recovery of WP was explained because of the improved whey retention. The parallel improvement of the rheological properties was measured in comparison to untreated control cheese, throughout ripening.

Gelation of proteins via acid coagulation is another way of cheesemaking. TGase treatment of cheese milk promotes the development of novel physical and functional properties of acid gels and the increase of cheese yield by improved whey recovery (Mazuknaite, Guyot, Leskauskaite, & Kulozik, 2013). In this case, TGase treatment was applied for the production of fresh and unripened cheeses, such as Quark and Cottage cheese (Kuraishi, Yamazaki, & Susa, 2001). To date, different patents on TGase application in cheesemaking to favor the enrichment in WP are available (Han, Pfeifer, Lincuort, & Schuerman, 2003; Han and Spradlin, 2000). Mazuknaite et al. (2013) evaluated conditions of milk incubation with TGase to optimize cross-linking of proteins. Increase of enzyme concentrations (from 1 to 4 U per g), incubation time (from 20 to 60 min) or incubation temperature (from 40 to 50 °C) expanded the degree of polymerization and changed the properties of the protein network formed during fermentation. Such acid gels, used to make Cottage-type cheese, allowed to increase the yield and improve textural properties by higher cohesiveness and lower gumminess values. However, proper pre-treatment conditions with TGase were necessary to avoid deterioration of the textural properties of Cottage-type cheese, due to excessive protein cross-linking.

While scientific interest has grown rapidly, the commercial application of TGase treatment of cheese milk is not a realistic processing alternative of interest.

Conclusions

The enhancement of WP content in cheese through either the adoption of techniques favoring WP retention in the cheese network or the direct addition of dairy-based ingredients or their combination is a challenging area in dairy sector. This topic is mainly targeted to increase cheese yield and to develop cheese varieties with novel texture and functional attributes, rather than duplicating conventional cheeses. Some techniques have been implemented and emerged in industrial processes, becoming routine steps for the inclusion of WP in cheese. Other solutions remained untapped due to their reduced cost-effectiveness and/or failure on quality attributes of cheese.

Perspectives for the implementation of cheese fortified in WP are supposed to be conditioned by various factors, namely: i) the advancement in basic research to deepen the understanding of the functionalities of WP assemblies in a real food system, such as cheese; ii) the improvement and scale-up of existing technologies; iii) the selection of proper adaptations in cheesemaking processing to overcome undesired drawbacks; iv) the proper integration in production lines of different techniques to enlarge the range of applications (e.g. membrane processes in multiple steps, etc.).

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Figure Captions

Fig. 1 Schematic overview of tasks related to the inclusion of whey proteins in cheese.

Fig. 2 Pre-treatments of cheese milk and main sources of protein-based derivatives to fortify cheese with whey proteins. WP: whey proteins; MWP: microparticulated whey proteins; WPC: whey protein concentrate; WPI: whey protein isolate.

Table 1 – Recent reports on milk pre-treatments to include whey proteins in cheese. Impact on cheese yield and overall properties.

Cheese	Milk pre-treatment	Processing conditions	Cheese yield[c] (*vs* control)	Cheese properties (*vs* untreated control)	Reference
Reduced-fat Cheddar with EPS-Ll[d]	UF[a]	VRF[b] = 1.2	Increased	Improved textural, melting and viscoelastic properties. Reduced bitterness.	(Agrawal and Hassan, 2007)
Semi-hard pressed cheese	UF	Total solid content of retentate from 36 to 41%	Up to + 2%	Improved quality by optimization of different technological parameters.	(Delgado, Salazar, & García, 2013)
Low-fat Camembert-type cheese	UF	VRF = 6	Increased	Delayed onset of proteolysis. Occorrence of surface defects.	(Hannon, Lortal, Tissier, & Famelart, 2009)
Half-fat Cheddar	Pasteurization	72 to 87 °C for 26 s	Increased	Decreased fracture stress, firmness, flowability and stretchability. Increase of viscosity.	(Rynne, Beresford, Kelly, & Guinee, 2004)
Urfa	Heat treatment	65 °C for 20 min 75 °C for 5 min	+ 3.5% at 85 °C + 9.9% at 90 °C	Severe heating impaired chemical and sensory properties.	(Atasoy, Yetişmeyen, Türkoğlu, & Özer, 2008)
Cottage	Heat treatment	85 °C for 5 min 90 °C for 5 min + 0.02% $CaCl_2$	+ 8% at 85 °C + 17% at 90 °C (*vs* cheese from pasteurized milk)	Texture and flavor unaffected.	(Makhal, Kanawjia, & Giri, 2015)
Low-fat Iranian white cheese	Cream homogenization (two-stage)	6.0–2.5 MPa and 9.0–2.5 MPa	Increased	Improvement of texture, flavor and appearance by homogenized cream.	(Madadlou, Mousavi, Khosrowshahi, Emam-Djome, & Zargaran, 2007)
Queso Fresco	HHP[e]	400 MPa for 20 min at 20 °C	Increased	Similar composition and texture properties. Less firm, less crumbly and more sticky.	(Sandra, Stanford, & Goddik, 2004)
Cheddar (65-day-old)	HHP	483 MPa for 5 min at 10 °C	+ 11.5% (*vs* cheese from pasteurized milk)	Treatment at 10 °C positively affected cheesemaking properties and rheological parameters.	(San Martin-González et al., 2007)

Cheddar (1-day-old)	HHP	400 and 600 MPa for 10 min at 20 °C	+ 1.2% at 400 MPa + 7.8% at 600 MPa	Increased cheese whiteness. Rennet coagulation time decreased at 400 MPa and increased at 600 MPa.	(Voigt, Donaghy, Patterson, Stephan, & Kelly, 2010)
Camembert	HHP	500 MPa for 10 min at 20 °C	Increased	Altered composition and accelerated hydrolysis of α_{s1}-casein. Similar sensory acceptability.	(Voigt, Patterson, Linton, & Kelly, 2011)
Full-fat Cheddar	UHPH[f]	200 MPa at 28 °C	+ 4.4% (*vs* cheese from pasteurized milk)	Firmer, more elastic and cohesive (*vs* cheese from pasteurized milk). Lowered microbial population.	(Kheadr, Vachon, Paquin, & Fliss, 2002)
Crescenza	UHPH	100 MPa	+ 1% (*vs* cheese from pasteurized milk)	Modified sensory and structural characteristics (*vs* cheese from pasteurized milk). No detrimental effects on shelf-life and safety. Early and significant lipolysis.	(Lanciotti et al., 2004)
Caciotta	UHPH	100 MPa at 5−7 °C	+ 23% (*vs* cheese from raw milk) + 32% (*vs* cheese from pasteurized milk)	Extensive proteolysis and volatile profile modified. Microbial population decreased (*vs* cheese from pasteurized milk).	(Lanciotti et al., 2006)
Pecorino-like cheese	UHPH	100 MPa at 5 °C	+ 23% (*vs* cheese from thermized milk) + 38% (*vs* cheese from raw milk)	Accelerated ripening with different proteolytic and lipolytic patterns. Different volatile profile.	(Vannini et al., 2008)
Starter-free fresh cheese	UHPH	300 MPa at 30 °C	Increased (*vs* cheese from pasteurized milk)	Less syneresis during storage and lower levels of lipolysis and proteolysis. Longer shelf-life. Occurrence of slight lipid oxidation.	(Zamora, Juan, & Trujillo, 2015)
Semi-hard goat cheese (1-day-old)	UHPH	200 MPa at 30 °C	+ 0.5% (*vs* cheese from homogenized-pasteurized milk) + 2% (*vs* cheese from pasteurized milk)	Higher hydrolysis of α_{s1}- and ß-casein (*vs* cheese from pasteurized milk). Lower hydrolysis of α_{s1}- and ß-casein (*vs* cheese from homogenized-pasteurized milk).	(Juan, Zamora, Quevedo, and Trujillo, 2016)

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[a] ultrafiltration.

[b] volume reduction factor.

[c] actual or moisture-adjusted yield.

[d] milk inoculation with exopolysaccharide-producing *Lactococcus lactis* ssp. *Cremoris*.

[e] high hydrostatic pressure.

[f] ultra-high pressure homogenization.

Table 2 – Recent reports on cheeses manufactured with milk fortification with dairy protein-based derivatives or buttermilk. Impact on cheese yield and overall properties.

Cheese variety	Added derivative	Milk-in-vat	Cheese yield (*vs* control, derivative-free)	Cheese properties (*vs* control, derivative-free)	Reference
Reduced-fat Cheddar	MPC63[a] (high WP denaturation)	S[b] to 5.3% casein	Doubled[c]	Reduced primary and secondary proteolysis. Starter adjuncts required to enhance ripening and flavor development.	(Ur-Rehman, Farkye, Considine, Schaffner, & Drake, 2003)
Pizza cheese	MPC64	S to 5.0% protein	+ 61%	Improved meltability during storage in Pizza cheese made by direct acidification.	(Ur-Rehman, Farkye, & Yim, 2003)
Cheddar	MPC85	S to 4.6% protein or S to 6.5% protein	Increased	No sensory defects in cheese from milk standardized to 4.6% in protein. Lower fat retention and adverse effects on texture in cheese from milk standardized to 6.5% in protein.	(Harvey, 2006)
Feta and Mozzarella	MPC85	S to 5.4% protein	Increased	No sensory defects.	(Harvey, 2006)
Cheddar	MPC84	S to 3.6% protein or S to 4.0% protein	+ 11% + 23%	Not investigated.	(Guinee, O'Kennedy, & Kelly, 2006)
Mozzarella	MPC83	S to 4.0% protein	+ 21%	Improved fat recovery by adapting stretching procedures. Positive evaluation by untrained panel of consumers.	(Francolino, Locci, Ghiglietti, Iezzi, & Mucchetti, 2010)
Iranian white cheese	Fermented WPC[d]	+ 5, + 10 or + 20% fermented WPC	Increased	Softer texture. Decreased hardness and chewiness. Impaired overall quality when fermented WPC >15%.	(Jooyandeh, 2009)
Low-moisture part-skim Mozzarella	WPC55	+ 0.25% WPC	Recovery of denatured WP	Reduced melting performance by cross-linked WP. Reduced fines production during shredding.	(Banville, Morin, Pouliot, & Britten, 2013)
Low-fat Cremoso Argentino	MWP[e] (Dairy-Lo™)	+ 1% MWP	Increased moisture	Extensive softening after 30 d of ripening. Low scores in taste and aroma. Similar meltability. Good quality without Dairy-Lo™.	(Zalazar et al., 2002)

Low-fat white pickled cheese	MWP (Simplesse®) or (Dairy-Lo™)	+ 0.5% MWP	Increased moisture	Hardness and sensory properties similar. Acceptable quality. Absence of off-flavor or bitterness.	(Kavas, Oysun, Kinik, & Uysal, 2004)
Low-fat fresh Kashar cheese	MWP (Simplesse® D-100) (Dairy-Lo™)	+ 1% MWP	Increased moisture	Improved flavor, texture and acceptability. Simplesse® D100 reduced hardness. Appearance defects corrected with Simplesse® D-100. Decrease of sensory scores over 60th day of storage.	(Koca & Metin, 2004)
Reduced-fat semi-hard cheese (Gouda-type)	MWP concentrate (CreamoProt®)	+ 0.5, + 1 or + 2% MWP	Increased	Improvement of texture attributes was concentration-dependent. Changed meltability.	(Schenkel, Samudrala, & Hinrichs, 2011)
Fresh cheese	MWP (liquid)	+ 25 or + 50 g MWP per 100 g milk protein	Increased	Lower syneresis and higher stability over storage. Reduced sensorial acceptability at higher levels of added MWP.	(Henriques, Gomes, Pereira, & Gil, 2013)
Reduced-fat Gouda-type cheese	MWP (Simplesse®)	+ 1% MW	Increased moisture	Improved thermo-physical properties. Reduced hardness. MWP acted as inert fillers in the melting cheese matrix.	(Schenkel, Samudrala, & Hinrichs, 2013)
Low-fat Caciotta-type cheese	MWP concentrate	+ 0.5% MWP + EPS-St[f] + cultures[g]	+ 33%	Improved textural properties. Overall acceptability Sensory scores similar to full-fat variant.	(Di Cagno et al., 2014)
Low-fat Beyaz pickled cheese	MWP (Simplesse®)	+ 1% MWP	Increased moisture	Enhanced texture properties. Sensory scores similar to full-fat cheese.	(Akin & Kirmaci, 2015)
Pizza cheese	Buttermilk condensed by heat and vacuum	+ 4 or + 6% condensed buttermilk	+ 10 or + 15%	Decrease of melt and stretch attributes.	(Govindasamy-Lucey, Lin, Jaeggi, Johnson, & Lucey, 2006)
Pizza cheese	Buttermilk UF[h]	Blend of retentate plus other ingredients	+ 11%	Structural and flavor attributes acceptable through adaptations. Slightly lower oiling off.	(Govindasamy-Lucey et al., 2007)
Reduced-fat Paneer	Buttermilk UF	+ 20, + 30 or + 40% retentate	+ 2%	Improved sensory attributes.	(Suneeta, Bhatt, & Prajapati, 2014)

[a] milk protein concentrate with xx% protein.
[b] standardization.

[c] actual or moisture-adjusted yield.
[d] whey protein concentrate.
[e] microparticulated whey proteins.
[f] milk inoculation with exopolysaccharide-producing *Streptococcus thermophilus*.
[g] milk inoculation with adjunct cultures.
[h] ultrafiltered retentate.

Table 3 – Recent reports on combined pre-treatments of milk to include whey proteins in cheese. Impact on cheese yield and overall properties.

Cheese	Milk pre-treatment	Processing conditions	Cheese yield (*vs* control)	Cheese properties (*vs* control cheese)	Reference
Raclette	Pasteurization + MF[a]	80 to 85 °C for 30 s + MF up to 17% of total solids	Increased	Good melting properties.	(Thomet, Bachmann, & Schafroth, 2004)
Gouda	Pasteurization + MF + Pasteurization	80 to 85 °C for 30 to 60 s + VRF[b] = 1.5 and 2.0 + 72 °C for 15 s	Increased	Heat-induced delay in rennet coagulation compensated by MF, and by adjusting coagulant and $CaCl_2$ addition. Sensory quality and behavior during industrial slicing satisfactory and according to specification.	(Chromik, Partschefeld, Jaros, Henle, & Rohm, 2010)
Starter-free fresh cheese	Two-stage Homogenization + Pasteurization	15−3 MPa at 57 to 60 °C + 72 °C for 15 s	Increased	Firmer cheese, less deformable and whiter color. Increase in bound water, but less water-mouth feeling.	(Zamora, Ferragut, Juan, Guamis, & Trujillo, 2011)
Queso Fresco	Heat treatment + UHPH[c]	65 °C for 30 min + 100 to 300 MPa	+ 1% each 100 MPa	Improved retention of sweet whey.	(Escobar et al., 2011)
Low-fat Norvegia cheese (Gouda-type)	MWP[d] + buttermilk	+ 3 or 6% MWP and + 15% buttermilk	Increased moisture	Recipe with 3% MWP and 15% buttermilk reduced firmness, improved texture and liking scores. Texture defects with + 6% MWP addition.	(Skeie, Alseth, Abrahamsen, Jihansen, & Øyaas, 2013)

[a] microfiltration.
[b] volume reduction factor.
[c] ultra-high pressure homogenization.
[d] microparticulated whey proteins.

Table 4 – Technological tools to enhance the content of whey proteins in cheese and consequences in terms of cheese characteristics.

Cheese milk pre-treatment	Pros	Cons
Heat	- Yield increase in some fresh acid cheeses. - Cheap technology.	- Impairment of rennet-induced coagulum in semi-hard and hard cheeses. - Reduced whey syneresis. Excessive softness and brittleness. - Possible delayed maturation and adverse effects on texture and flavor.
UF[a]	- Yield increase. - Industrial adoption for manufacturing of several UF-cheese varieties.	- Possible structure and flavor defects without proper cheesemaking adjustments.
HHP[b]	- Yield increase. - Possible duplication of conventional cheese. - Novel texture and sensory quality. - Accelerated proteolysis and lipolysis. - Extended shelf-life of fresh cheeses.	- Possible altered composition with modifications of structure and volatile profile. - High investment, operation and maintenance costs. - Long treatments.
UHPH[c]	- Yield increase. - Ripening acceleration by more extensive proteolysis and lipolysis. - Longer chemical and microbiological shelf life. - Novel texture and sensory quality.	- Possible textural and sensorial modifications. - Possible lipid oxidation. - Additional equipment required.
Addition of protein-based derivatives	- Yield increase. - Reduced defects of low-fat cheeses and improved sensory attributes. - Microparticulated whey proteins used also to control moisture and texture of certain cheeses.	- Slight texture modification (softer curd). - Possible modification of ripening and flavor development. - Possible bitter defects by denatured whey proteins.
Addition of buttermilk or derivatives	- Yield increase. - Moisture increase.	- Adaptations in cheesemaking protocol to mantain functional properties of the cheese. - Recovery in whey proteins dependent on composition of buttermilk and processing conditions of the cream.

TGase[d]	- Yield increase. - Fresh and unripened cheeses with reduced wheying-off during storage. - Upon acid coagulation novel physical and functional properties.	- Limited industrial applications due to the impairment of rennet coagulation properties. - Changes in cheesemaking protocol to keep cheese acceptability.
Combined processes	- (Heat + UF) treatments of milk restore coagulation aptitude. - (UHPH + heat) treatments of milk increase yield and improve textural quality of fresh cheeses. - (Heat + TGase) treatments of milk increase yield and improve textural properties of cheese obtained by acid coagulation. - (UF + buttermilk) or (evaporation + buttermilk) in the manufacture of high-moisture cheeses.	- Adaptations in cheesemaking protocol.

[a] UF = ultrafiltration.

[b] HHP = high hydrostatic pressure.

[c] UHPH = ultra-high pressure homogenization.

[d] TGase = transglutaminase.

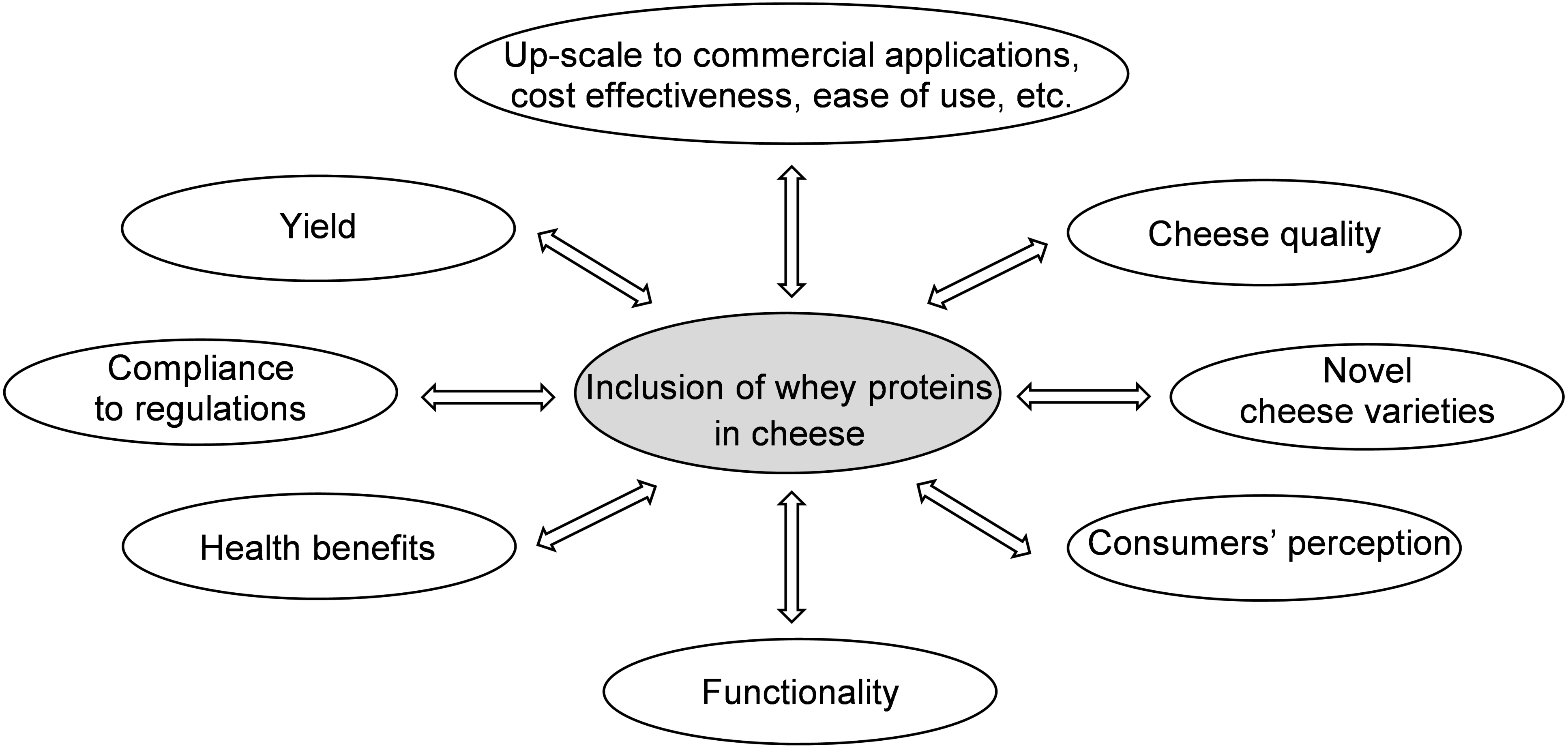
Up-scale to commercial applications, cost effectiveness, ease of use, etc.
Yield
Cheese quality
Compliance to regulations
Inclusion of whey proteins in cheese
Novel cheese varieties
Health benefits
Consumers' perception
Functionality

Fig. 1

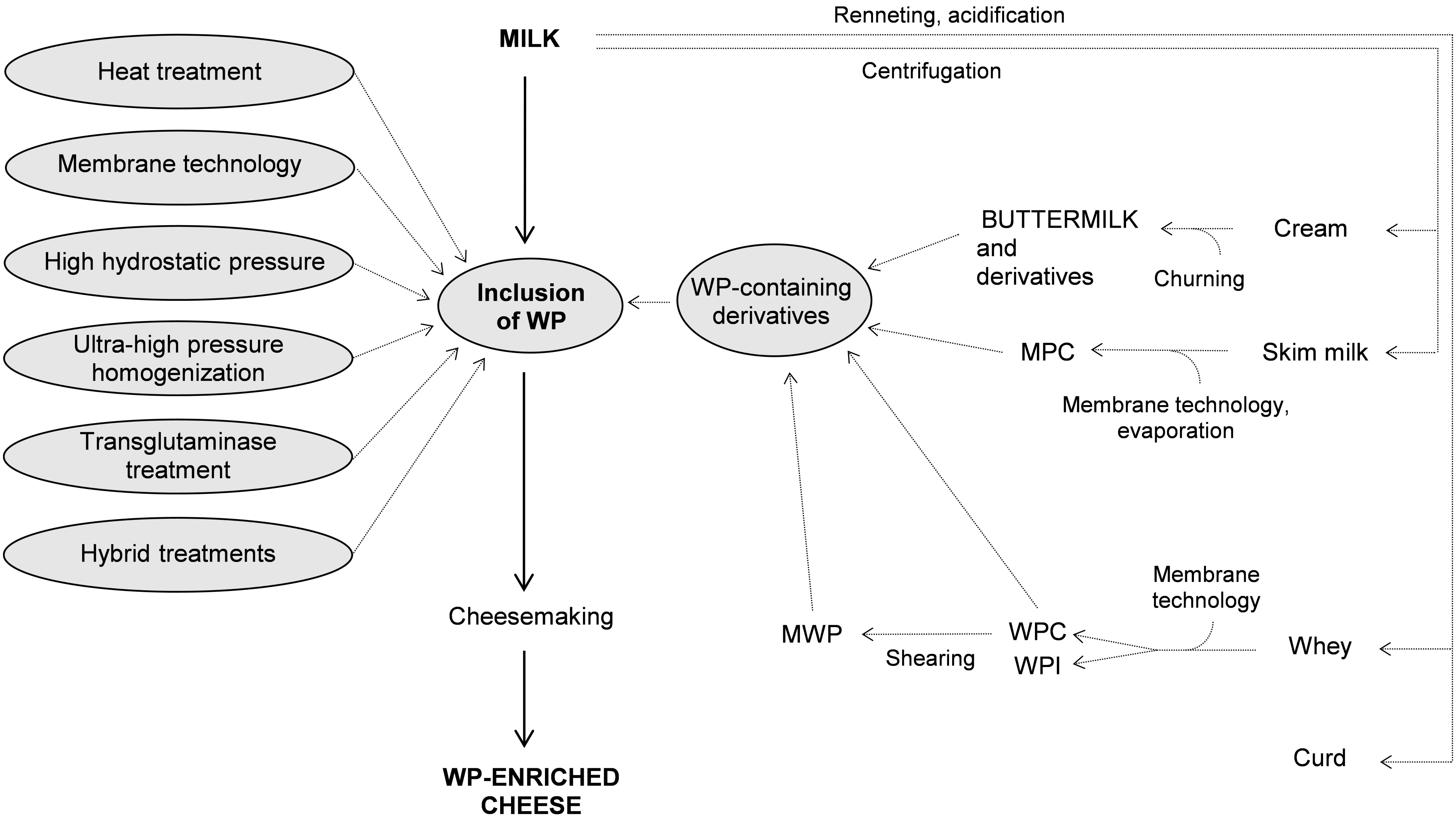
Renneting, acidification
MILK
Centrifugation
Heat treatment
Membrane technology
High hydrostatic pressure
Ultra-high pressure homogenization
Transglutaminase treatment
Hybrid treatments
Inclusion of WP
WP-containing derivatives
BUTTERMILK and derivatives
Churning
Cream
MPC
Skim milk
Membrane technology, evaporation
Membrane technology
MWP
Shearing
WPC
WPI
Whey
Curd
Cheesemaking
WP-ENRICHED CHEESE

Fig. 2

HIGHLIGHTS

- Valorization of whey proteins upon their inclusion into cheese
- Feasibility of several technological tools at industrial-scale or pilot-plant level
- Adaptations of manufacturing process to keep overall quality of the cheese
- Cheese varieties with novel functionalities following inclusion of whey proteins